OFFICE OF NAVAL RESEARCH

Contract N00014-67-A-0399-0009

Task No. NR 136-916

ANNUAL REPORT NO. 1

Ecological Relationships Between Arboviruses, Ectoparasites, and Vertebrates in Ethiopia

by

George E. Watson, Principal Investigator

Department of Vertebrate Zoology
National Museum of Natural History
Smithsonian Institution
Washington, D. C. 20560

and

John S. Ash, Co-investigator

Department of Vertebrate Zoology
National Museum of Natural History
Smithsonian Institution
Washington, D. C.20560

c/o NAMRU-3 Field Facility,
Ethiopia
APO New York,N.Y. 09319

31 August 1972

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY *(Corporate author)*

Smithsonian Institution
Washington, D.C. 20560

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP
NA

3 REPORT TITLE

ECOLOGICAL RELATIONSHIPS BETWEEN ARBOVIRUSES, ECTOPARASITES, AND VERTEBRATES IN ETHIOPIA.

4 DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

Annual Report (September 1, 1971 through August 31, 1972)

5 AUTHOR(S) *(First name, middle initial, last name)*

George A. Watson
John S. Ash

6 REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
31 August 1972	17	1

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
N00014-67-A-0399-0009	
b. PROJECT NO. NR 136-916/8-16-71	
c.	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	

10 DISTRIBUTION STATEMENT

This document has been approved for public release; its distribution is unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Department of the Navy Office of Naval Research Arlington, Virginia 22217

13 ABSTRACT

The primary aim of the project is to identify the natural vertebrate hosts of arboviruses infecting man in Ethiopia, through the collection and identification of animal species inhabiting areas where the viruses are endemic, and the determination of the immunological status of the material. The relative ecological importance of infected vertebrates is being assessed. Five study areas have been established from which approximately 33,500 animals have been captured. Over 9,000 sera have been tested against three Group B arbovirus hemagglutinating antigens. A detailed analysis of the bird sera shows that there is no major difference in over-all antibody rates between the five areas and that species of birds in eight families provide results which are to be pursued in greater detail; adequate samples are available from six species in eight families which show that they are not reservoir or amplifying hosts of Group B arboviruses. Among other groups there is a high incidence of Group B antibody in a monkey, a baboon, two fruit bats and a lizard. Rodents and amphibia are of little or no importance.

Population estimates are being obtained for each species in each area; over 15,000 birds have been banded to provide information on movements and survival; over 5,000 blood smears have been collected for a hemoparasitological survey; a collection of ectoparasites have been made; and a grid mapping scheme has been developed to plot the detailed distribution of animals, Group B arboviruses, and vectors in Ethiopia.

DD FORM 1 NOV 65 1473

Security Classification

14 KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Arbobiruses						
Ethiopia						
Vertebrates						
Ectoparasites						
Ecological Relationships						
Birds						
Mammals						
Reptiles						
Amphibians						
Ticks						
Blood smears						
Hemoparasites						

ECOLOGICAL RELATIONSHIPS BETWEEN ARBOVIRUSES, ECTOPARASITES, AND VERTEBRATES IN ETHIOPIA

INTRODUCTION

With few exceptions, the natural vertebrate hosts of the arboviruses are wild animals. The range of hosts includes many terrestrial and arboreal mammals, birds, reptiles and amphibia. Unfortunately, because of the limited geographic distribution of many of the viruses, and the expense of intensive long-term field investigations, the natural hosts and infection cycles have not been identified for many of the viruses in this group. Moreover, information derived from studies of one ecosystem may not be applicable to another, where the topography, climate, fauna and flora are quite different. Thus, there is great need for continued work on arbovirus-host-vector relationships, not only to unravel the epidemiological and epizootiological features of the disease, but also for the practical object of protecting man from infection through control of the important animal-to-man vectors.

Serologic surveys have revealed an extensive arbovirus distribution in Ethiopia, with antibody rates being particularly high in residents of the western lowlands of Illubabor Province and the valleys of the Didessa, Blue Nile, Awash, and Omo Rivers. Antibody patterns point to the presence in these areas of viruses belonging to the A, B, and Bunyamwera groups, and the high rate of plurally-reactive sera suggests that each group may be represented by several agents. To date, four Group B viruses (yellow fever, West Nile, Zika and Ntaya) have been recovered from animal and arthropod sources in Ethiopia, but isolation

studies have not been sufficiently intensive to reveal which members of the other two groups are present. However, it is reasonable to expect, by virtue of their prevalence in neighboring countries, that chikungunya, o'nyong-nyong, Sindbis, Ilesha and Germiston viruses will be found to be endemic in Ethiopia. The presence of such a variety of medically important arboviruses in a limited geographical area affords an ideal opportunity to extend work on their natural host ranges and, through the application of quantitative ecological methods, to identify those factors contributing to their maintenance in nature.

OBJECTIVES

As outlined in the original project proposal, the primary aims of the study are:

1. To identify the natural vertebrate hosts of arboviruses infecting man in Ethiopia, through:
   a. systematic collection and identification of animal species inhabiting endemic areas;
   b. determination of immune status of the material collected.
2. To assess the relative importance of naturally infected vertebrates as virus disseminators, through:
   a. quantitations of population densities;
   b. determination of host attractiveness to arthropods known to be naturally infected;
   c. estimation of population "turnover" rates and their significance in providing a continual pool of susceptibles;

d. determination of the level and duration of viremias resulting from peripheral inoculation of virus.

Secondary benefits deriving from the work include:

1. Information on the geographic distribution of mammals, birds and reptiles in Ethiopia.
2. Data on animal dispersal and migration, feeding habits, behavior towards traps, and the localization and characterization of microhabitats.
3. Information on the influence of climatic conditions, altitude and vegetation on animal distribution.

PLAN OF OPERATIONS

1. Selection of five study areas which the results of the human serological survey have shown to be most important in Ethiopia (see below).
2. The establishment of liaison with virologists (for serological survey) and with entomologists (for vector studies).
3. Broad ecological survey of each area.
4. Development of trapping techniques.
5. Development of recording systems.
6. The establishment of arrangements for the determination of unidentified material.
7. Detailed serological survey of the animals in each of the five areas, so that the next phases of the project could be planned. Emphasis was placed on birds - about which more

is known and which can be caught more easily. The aim was to sample 50 individuals of each species.

8. Record distribution and status of each species.
9. The establishment of a marking system (e.g. bird banding for individual recognition, life data, dispersal, etc.
10. Collection of ectoparasites.
11. The development of a country-wide mapping scheme for plotting the distribution of host species, vector hosts, etc.

Although there were inherent difficulties in drawing up a projected plan for what is essentially and initially a survey, an attempt was desirable. This, however, had to be fluid in order to enable promising lines of inquiry - based on new findings - to be taken up.

By its very nature this is a long-term project, and there is difficulty in close-ending it at an intermediate stage. The basic serological survey, upon which the rest of the program must be based, will take much longer than was originally envisaged. The results of intensive field work in the project's first year indicated that it would take at least 5 years to obtain adequate samples of the more abundant species in each area.

In general terms, a five-year program from September 1971 was designed to cover the following topics:

A. Continuation of the field collections and serological testing (years 1971/1972).
B. Depending on the results of the serological survey, an attempt to obtain direct evidence for natural infection through re-

covery of virus from selected animal species (years 1972/1975).

C. Detailed work on the ecology and habits of any animals proven to be epidemiologically important (years 1973/1976).

D. Detailed investigation of host-vector relationships in elucidating natural infection cycles (year 1974/1976).

METHODS

Study Areas:

The five sites selected are as follows:

1. Gambela (8°15'N, 34°35'E), Ilubabor Province, on the Baro River in the western lowlands; riverine marsh and grassland adjoining Combretum/Terminalia woodland; altitude: 515 m.
2. Didessa (9°02'N, 36°09'E), Wollega Province, tropical deciduous woodland between riverine forest and open savannah; altitude: 1,200 m.
3. Bahadu (10°05'N, 40°37'E), Harrar Province, on the Awash River in the Danakil desert, lacustrine flood plain; altitude: 600 m.
4. Bulcha Forest (6°27'N, 38°11'E), Sidamo Province, riverine forest near Lake Margherita adjoining open acacia savannah; altitude: 1,320 m.
5. Abiata/Koka (7°36'N, 38°40'E - 8°27'N, 39°06'E), Shoa Province, two sites in lakeside acacia savannah in the Rift Valley; altitude: 1,590 m.

Trapping:

Birds have been caught almost exclusively in mist nets. Successful capture of many species depends upon an intimate knowledge of their feeding and habitat preferences and their habits. Success with elusive species continues to improve with experience. Bats are all caught at night, also with mist nets, and this method has proved to be much more productive than the time-consuming method of searching for their diurnal quarters. Mammals are caught in baited cage traps or are shot. The possibility of using rocket-propelled nets for the capture of large water birds is being investigated.

Collection of blood:

Blood is obtained by cardiac puncture or from the jugular vein and separated in the field. Sera are refrigerated until return to the laboratory.

Serology:

The hemagglutination-inhibition (H-I) test is used for screening, and the neutralization test for specific confirmatory tests when volumes of sera are sufficient. When H-I test results suggest infection with a single virus, H-I positive serum pools will be used in neutralization tests to confirm the identity of the infecting agent.

RESULTS

Progress towards fulfilling each of the objectives is summarized here.

Specimen collection and identification

A total of approximately 33, 500 birds and other animals has been captured in the five study areas (Table 1). Nearly 2500 bird skins, 650 bats, 250 other mammals and 400 reptiles and amphibia have been prepared as study specimens. Identification keys have been prepared for some groups of birds, rodents, bats and amphibia, for which adequate keys did not previously exist in the literature.

TABLE I
Summary of animals captured in the five study areas in Ethiopia: November 1969-June 1972

Captured	Bulcha	Gambela	Didessa	Bahadu	Rift Valley
Birds	1650	4879	2902	6092	15, 757
Mammals	146	544	371	140	297
Reptiles/ Amphibia	65	509	102	19	5
Totals	1861	5932	3375	6251	16, 059

Serology

A total of 9035 sera has been collected, and all of these have been tested against 3 Group B arbovirus hemagglutinating antigens. The results from the birds have now been analyzed in detail, and work is proceeding with the other groups.

Results from the bird sera indicate that:

1. There is no major difference in overall Group B antibody rates between the five areas (mean 3.8%; range 2.9 - 5.0%).

2. Special attention should be paid to Bahadu and Bulcha where the antibody rates are highest, and to Gambela in collaboration with the two surveys being undertaken there by NAMRU on "Acute febrile illnesses" and the isolation of viruses from mosquitos.

3. Within "positive species" (i.e. those for which a serum sample in the H-I test in a 1:20 dilution inhibits hemagglutination by at least one of the three antigens used) the small samples involved do not permit drawing conclusions regarding differences in antibody rates between the five areas.

4. Species of birds within the following families provide results which indicate that they should be followed up in greater detail:

   Ardeidae (Butorides striatus), Falconidae, Phasianidae, Columbidae (Streptopelia semitorquata and decipiens, Oena capensis, Turtur afer, Treron waalia), Upupidae (Upupa epops), Turdidae (Turdus pelios and olivaceus, Cercomela familiaris), Laniidae (Dryoscopus gambensis, Tchagra senegalensis), Ploceidae (Sorella eminibey).

5. Adequate serum samples have been tested from the following families to show that they are not amplifying or reservoir hosts of Group B arboviruses: Jacanidae, Charadriidae, Scolopacidae, Pycnonotidae, Zosteropidae, Nectariniidae, Ploceidae (4 species), Fringillidae.

A detailed examination now being undertaken of the results obtained from the sera of other groups (mammals, reptiles and amphibia) indicates that:

1. There is a high rate of Group B antibody in the following species or groups: a monkey Cercopithecus aethiops, a baboon Chaeropithecus doguera, two fruit-bats Epomophorus labiatus and Micropteropus pusillus and Agama lizards.
2. Rodents and amphibia are of little or no importance in the maintenance of Group B arboviruses in the study areas.

Neutralization tests on selected sera have been started, and will continue. The first batch of ten Streptopelia decipiens sera in which the H-I antibody rates were high, have been tested against West Nile and Zika with negative results suggesting past infection with a different Group B virus. Further neutralization tests will be made against other agents.

Population estimates

Through a system of daily census, population densities of the vertebrate fauna in each of the study areas are being estimated.

"Turnover rates"

The banding of over 15,000 birds is providing information on "turnover" rates and movements in the five areas. There is good evidence for normal survival rates of bled birds.

Geographic distribution

A National Mapping Scheme has been developed to plot the distribution of mammals, birds, reptiles and amphibia in Ethiopia. The ultimate aim is to complete distribution overlay maps for the Group B arboviruses, natural wild hosts and vectors. This scheme has been adopted by other divisions within NAMRU, and by other organizations

and agencies within the country, in connection with distributional work on a wide range of organisms, including insect and fungal pests, game animals, plants, etc. (Ash, 1972).

Publications

Nine papers arising from the results of this work have been completed or nearly so for submission to the designated Journals.

Ash, J.S. 1972. Distribution Map Scheme for Ethiopia. Ibis, 114:109.

Further evidence for Orstreue from Ethiopia (Bird Study).

Six species of birds new to Ethiopia (Bulletin British Ornithologists' Club).

Luscinia luscinia and L. megarhynchos in Ethiopia (Ibis).

The 'Boran" Cisticola in Ethiopia (Ibis).

Streptopelia reichenowi in Ethiopia (Ibis).

Charadriform birds in the Ethiopian Rift Valley (Walia).

A migration of Palearctic birds inland in Ethiopia (Ibis).

Autumn migrants in the Cherchers and Ogaden, Ethiopia (Ibis).

Blood Smear and Ectoparasite Collections

Over 5,000 blood smears have been collected for a hemoparasitological survey, and the results are being prepared for publication. Ectoparasites, particularly ticks, are collected for Dr. Harry Hoogstraal for the survey of African tick-borne diseases at NAMRU-3, Cairo.

Plan of Work for 1973-1976

The serological survey upon which the rest of the program must be based will take at least another four years to obtain adequate samples of the more interesting species in each area. The following tentative timetable of work is proposed for the four years:

1. Continuation of the field collections and serological screening by H-I test (1973 onwards), with greater emphasis on the three more interesting areas.
2. The use of neutralization tests on selected sera to identify the specific infecting virus (es). Existing facilities at NAMRU will enable about 250 serum specimens to be tested against 4-6 viruses per year (years 1973-1975).
3. An attempt to obtain direct evidence for natural infection through isolation of virus from selected animal species (1973-1975). Several species have now been selected for intensive study.
4. Detailed work on the ecology and habits of any animals proven to be epidemiologically important (years 1973-1976).
5. Detailed investigation of host-vector relationships in elucidating natural infection cycles (years 1974-1976).